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Exploration of the Energy Landscape of Protein-Protein and Antibody-Antigen Interactions

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A well choreographed, dynamic interplay of protein-protein interactions is crucial for the function of a cell. To understand these interactions, knowledge of the underlying energy landscape is essential. We analyzed the energy landscape of a protein-protein and an antibody-antigen complex using steered molecular dynamics simulations.

First, we examined the influence of velocity and geometry of the force probing on the choice of the forced unbinding pathway of the Barnase-Barstar complex. We demonstrated that in our constant velocity probing experiments, a change in the probing velocity may switch the unfolding pathway. Further more, we showed, that changing the geometry of the force probing can be used to choose between different unbinding pathways. These tools may be used for a pre-chosen sampling of the protein complex energy landscapes.

The second part of our work focused on the examination of the dependence of the unbinding pathway on the force attachment point. The truncated Leucine zipper GCN4 peptide was separated from the anti-GCN4-antibody fragment H6 in SMD simulations. Three different attachment points were examined: the C and N terminal C_αs of the 12 amino acid long peptide as well as a C_α in the middle of the peptide. We identified a common barrier on the unbinding pathway formed by a shared, central unbinding interaction.

Additionally, we classified the correlation between MD simulations and AFM as well as SPR measurements. We could show, that, in the examined system, the AFM probes the first barrier found in our MD simulations. Further more, our MD trajectories showed the existence of two main unbinding barriers. This supports the theory, that AFM and SPR may test different barriers. The second barrier is tested adiabatically with SPR measurements, while the inner barrier is probed via AFM, due to the forced tilting of the energy landscape inherent to force spectroscopy measurements.

1 Introduction

The multidimensional energy landscape of a biological molecular complex is an intrinsic property determining the dynamic function of the system. It can be described by barriers and energy minima. The barriers are blocking access to other regions of the energy landscape while the local minima define stable conformations of the complex. A system in thermodynamic equilibrium is preferentially in the global minimum of the energy profile. Disturbing the system enables sampling of the energy landscape[1,2,11]. According to the Bell-Evans model[3] the energy landscape can be tilted by applying forces. Therefore, the system is able to move over lower barriers to different regions of the energy profile. Here, we examined at the example of the Barnase-Barstar[4–7] complex[a] how the energy landscape

[a] One complex of the PDB structure 1BRS was used.

of a protein-protein complex can be more thoroughly explored. This is accomplished by using different force attachment points and different probing velocities[8]. To access larger regions of the energy landscape belonging to an antibody fragment complexed with its peptide antigen, dynamic force spectroscopy was performed by Steered Molecular Dynamics (SMD) simulations as well as Atomic Force Microscopy (AFM). To sample the equilibrium states, Surface Plasmon Resonance (SPR) was used[9].

2 Barnase Barstar Complex

2.1 Stability vs. Lability

In this experiment, we simulated the force application in analogy to AFM experiments, attaching the probing forces at single C_α-atoms[8,10]. Barstar was pulled at the last C_α of its C-terminal secondary structure element and Barnase was fixed at the first C_alpha of its N-terminal secondary structure element and vice versa. The velocity of force probing way changed from fast ($2\frac{m}{s}$ to slow ($0.5\frac{m}{s}$) pulling in these experiments, investigating the difference between probing the lability and stability of the system. If solely the system's lability is tested, it lacks time to relax in the changing energy landscape and follows the pathway of the lowest barrier heights. In case of probing only the stability of the system, the time for relaxations is sufficient and the pathway is determined only by the lowest energy. Due to the non-adiabatic pulling, the system lacks sufficient time to relax to the state of minimum energy. Hence, not only the well depth, but also the barrier height is determining the pathway. The influence of barrier heights against well depth increases with probing velocity.

2.2 Dependence on the Force Attachment Point

The differences in the anti parallel probing of the Barnase-Barstar complex lead to an extended exploration of the importance of the attachment point on the choice of the unbinding pathway. Here, typical COM measurements resulted in the unbinding of the complex only after Barstar's binding helix was separated from its protein core. Furthermore, we show, that a direct probing of the binding interface leads to a direct unbinding of the complex along two distinguishable pathways.

3 Antibody Antigen Interaction

To increase the understanding of antigen-antibody interactions, we explored the energy landscape of the antibody fragment H6 to its antigen GCN4-p1 Leucine zipper. Dynamic force spectroscopy experiments were conducted using AFM and SMD simulations to gain access to different velocity regimes. The equilibrium unbinding was determined via SPR. Probing different force attachment points on the peptide, we showed, that the unbinding under force depends on the direction of pulling, while adiabatic measurements only revealed one unbinding pathway.

The first SMD barrier rupture lengths agree with the potential widths measured by AFM. The second SMD barrier might resemble the barrier probed by SPR. These results

suggest that the system is dominated by a two barrier unbinding with a lower inner barrier and a higher outer barrier. AFM probes the inner barrier due to the applied forces tilting the energy landscape, while SPR adiabatically probes the higher outer barrier.

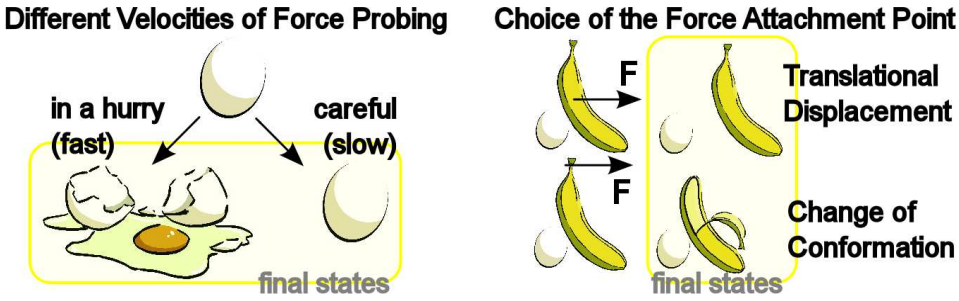

Figure 1. The exploration of the energy landscape with dynamic force spectroscopy depends not only on the choice of the attachment point of the forces, but also on the velocity of force probing. By choosing the attachment point and the probing velocity, the exploration of the energy landscape of a complex can be preferentially guided to certain regions.

4 Conclusion

The applied forces alter the energy landscape in a non-trivial way. The resulting propagation of the probed complexes through phase space does not only critically depend on the geometry of force application, but equally on the velocity of force application. Increasing the pulling velocity diminishes the time for the system to relax in the current energy landscape. The different attachment points of the force pulling alter the distribution of the tension in the complex. This results in probing different properties of the highly complex biological systems. Therefore, for a thorough characterization of the effect of force on a protein complex, multiple simulations with different probing geometries and different velocities need to be performed.

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